Unusual fatty acid composition of storage lipids in the bresilioid shrimp *Rimicaris exoculata* couples the photic zone with MAR hydrothermal vent sites

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ABSTRACT: Lipid and stable carbon isotope analyses of *Rimicaris exoculata*, the dominant bresilioid shrimp found at the MAR (Mid-Atlantic Ridge) vent sites, have indicated that these animals possess a highly unusual storage lipid composition. The dominant neutral lipid classes, triacylglycerols and wax esters, contained very high levels of polyunsaturated fatty acids (PUFA, up to 89% of neutral lipid fatty acids). Gas chromatography isotope ratio mass spectrometry (GC-IRMS) analysis of the PUFA from neutral lipid gave $\delta^{13}C$ (v-PDB) values of –17.6 to –27.1‰, which is within the range expected for a photosynthetic origin for these compounds. Fatty acid analyses of bacterial/detrital material collected from the vent sites contained only very low amounts of PUFA. It is clear from these findings that *R. exoculata* has evolved a highly specialized lipid metabolism which allows it to store substantial amounts of PUFA during its early planktotrophic life stages. These PUFA reserves will be subsequently mobilized to enable growth and maturation of the shrimp on return to a suitable vent site and are therefore an important factor allowing *R. exoculata* to inhabit deep sea vent ecosystems.

KEY WORDS: Hydrothermal vent shrimp · Nutrition · Lipid metabolism · Wax ester · PUFA

INTRODUCTION

The food sources of *Rimicaris exoculata*, the dominant shrimp inhabiting the Mid-Atlantic Ridge (MAR) hydrothermal vent sites, have been studied for a number of years. These studies have suggested that the shrimp derives its nutritional requirements from strains of chemoautotrophic bacteria that either encrust the walls of the vent chimneys, or encrust the carapace of the shrimp itself (Van Dover 1995, Gebruk et al. 1997 and references therein). More recently it has been found that bacterial populations within the gut of *R. exoculata* also exhibit high rates of chemoautotrophic production and could constitute an additional food source (Polz et al. 1998). However, for higher marine

animals, there are important nutritional limitations for a diet that almost exclusively comprises bacteria. Some marine organisms including decapod shrimp require certain essential polyunsaturated fatty acids (PUFA) to be supplied in their diet (Kanazawa et al. 1979). These compounds have crucial roles in the structure and functioning of cell membranes, and the animals require substantial amounts during periods of growth and reproduction (Jónasdóttir 1994, Sargent et al. 1995, Pond et al. 1996). In the marine ecosystem as a whole, the bulk of PUFAs is produced by phytoplankton (Sargent & Henderson 1995). Bacteria are generally incapable of synthesizing polyunsaturated fatty acids as they tend to lack the necessary oxygen-dependent desaturases. In recent years some strains of PUFA-producing heterotrophic bacteria have been isolated from a range of marine environments (Russell &

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Nichols 1999), but, as yet, none have been identified at MAR vent sites. It is therefore unlikely that the chemo-autotrophic bacteria on which *R. exoculata* feeds at the vent sites contain sufficient amounts of PUFA for the nutrition of this shrimp. The key question we examined here is how the populations of vent shrimp inhabiting the MAR vent sites have solved this apparent nutritional paradox.

Previous studies have shown that the early life stages of MAR vent shrimps can accumulate PUFAs derived from phytoplankton (Pond et al. 1997a,b,c). During these early stages the larval shrimp are components of the deepwater plankton community and their lipid composition is typical of bathypelagic organisms, which are exclusively reliant on organic carbon originating from the surface layers of the ocean (Pond et al. 1997a, in press). Here we extend the previous work of Pond et al. (1997a,b,c, Dixon et al. 1998) by analyzing the lipid and stable isotope composition of different developmental stages of *Rimicaris exoculata* from a variety of MAR vent sites.

METHODS

Sampling. Specimens of *Rimicaris exoculata* were collected by slurp-gun from 6 sites along the MAR during a cruise of RV 'Atlantis' and the submersible 'Alvin', when all known sites of hydrothermal activity were visited. Table 1 summarizes the position and depth of the sites where *R. exoculata* occurred. After arrival of shrimp on board ship, animals were quickly sized (total length) and identified in terms of 3 maturity stages. In the first category small 'orange' shrimp were between 16 and 18 mm in length, bright orange in color and contained substantial lipid reserves which were easily visible under the carapace. These shrimp were initially referred to as *Iorania concordia*, originally described as a separate species by Vereshchaka (1996). However, it is now established that these small orange shrimp are a juvenile form of *R. exoculata* (Shank et al. 1998). We analyzed 2 further categories of *R. exoculata*, i.e. small adults (25 to 26 mm), which

Table 1. *Rimicaris exoculata*. Locations of the Mid-Atlantic Ridge (MAR) vent sites

Site	Latitude N	Longitude W	Depth (m)
Lucky Strike	37° 17.5'	32° 16.5'	1600–1720
Rainbow	36° 14.0'	33° 54.0'	2305–2350
Broken Spur	29° 10.0'	43° 10.4'	3050–3060
TAG	26° 08.0'	44° 49.5'	3640–3660
Snakepit	23° 22.2'	44° 57.0'	3510–3560
Logatchev	14° 44.9'	44° 58.3'	3010–3038

were a whitish gray in color, and larger adults (29 to 57 mm). Neither the small nor larger adults contained the substantial pigmented lipid reserves found in the juveniles. Immediately after characterization, shrimp were dissected and the tissue samples transferred to chloroform:methanol (2:1 v/v) and stored at –20°C until analysis in the UK.

Lipid analyses. Samples of tissue contained in the chloroform:methanol were initially homogenized before being filtered through a prewashed (chloroform:methanol 2:1, v/v) Whatman No. 1 paper filter. Total lipid was extracted following Folch et al. (1957) and dried under nitrogen. Aliquots of total lipid (15 µg) were separated into individual lipid classes of neutral lipid by high performance thin layer chromatography (HPTLC) using a hexane:diethyl ether:acetic acid (90:10:1 v/v/v) solvent system. Lipid classes were visualized by spraying the plates with 8% (v/v) phosphoric acid containing 3% (w/v) copper acetate followed by charring at 160°C for 15 min and then quantified by scanning densitometry (Olsen & Henderson 1989).

Remaining samples of total lipid were separated into polar lipid, triacylglycerol and wax ester by thin-layer chromatography (TLC), as above, and visualized under UV light after spraying with 2,7-dichlorofluorescein in 97% (v/v) methanol. Individual lipid classes were then transesterified in methanol containing 1.5% (v/v) sulphuric acid for 16 h at 50°C, and the resulting fatty acid methyl esters were purified by TLC in a hexane:diethyl ether:acetic acid solvent system (90:10:1 v/v/v). For the wax ester fraction, free fatty alcohols were recovered separately from the TLC plates and converted to their acetate derivatives following Farquhar (1962). Purified fatty acid methyl esters and fatty alcohol acetate derivatives were analyzed separately by gas chromatography (GC) on a Carlo Erba (6000 vega series) fitted with a BP20 fused silica capillary column (50 m by 0.32 mm internal diameter). Hydrogen was used as the carrier gas. Fatty acids were identified by reference to standards of known composition and by GC-MS as described in Pond et al. (1997b, in press).

GC-IRMS analyses. Stable carbon isotope ratios ($^{13}C/^{12}C$) were determined for individual fatty acids and fatty alcohols by GC-combustion IRMS (isotope ratio mass spectrometry) with a VG Isochrom II instrument equipped with a column similar to that described in the preceding section (Eakin et al. 1992). The $\delta^{13}C$ value of the methanol derivatization reagent was determined by conventional closed-tube combustion (Sofer 1980), and the contribution of derivatized carbon to specific fatty acids was calculated by rearranging the equation from Abrajano et al. (1994):

$$\delta^{13}C_{FA} = \frac{\delta^{13}C_{FAME} - (1-x)\delta^{13}C_{CH_3OH}}{x}$$

where: $\delta^{13}C_{FA}$ is the isotopic composition of the free fatty acid, $\delta^{13}C_{FAME}$ is the isotopic composition of the fatty acid methyl ester, x is the fractional carbon contribution of the free fatty acid to the ester and $\delta^{13}C_{CH_3OH}$ is the isotopic composition of the methanol derivatization reagent. All isotope data are reported as $\delta^{13}C$ (‰ v-PDB) and precision is ± 0.4‰ or better.

RESULTS

Lipid class composition

The lipid class composition of the 3 categories of *Rimicaris exoculata* are summarized in Fig. 1. The lipids of the juvenile shrimp comprised predominantly wax esters (56% of total lipid) with only comparatively minor amounts of triacylglycerol (10%, Fig. 1). Small adult *R. exoculata* contained both wax ester and triacylglycerols in similar proportions (31 and 24%), whilst the lipid composition of adult *R. exoculata* was dominated by triacylglycerols and polar lipid (Fig. 1).

Fatty acid and fatty alcohol composition

Bacterial/detrital material collected from vent sites contained only very low proportions of PUFA (approx.
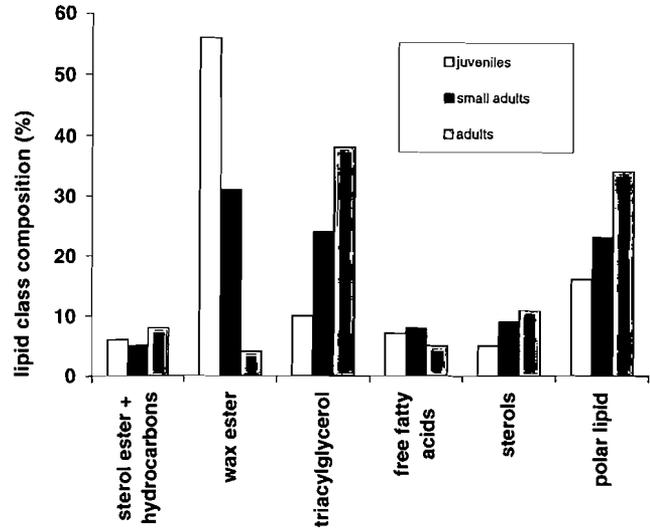

Fig. 1. *Rimicaris exoculata*. Lipid class composition (% wt). Values are means of all shrimp in each of the 'orange' juvenile (n = 4), small adult (n = 2) and adult (n = 6) categories

1%), comprising mostly saturated and monounsaturated fatty acids (Table 2). By contrast the fatty acid compositions of the juvenile *Rimicaris exoculata* were dominated by the polyunsaturated fatty acid 22:6(n-3), with wax ester consistently more enriched in this fatty acid than polar lipid (Table 3). Other major fatty

Table 2. Fatty acid composition (%) of total lipid extracted from filamentous free-living bacteria (fb) and detrital material (dm) collected from MAR vent sites (*collected from mussel shells; a, b, c refer to replicates)

Site:	Lucky Strike			Rainbow	Snake Pit	Logatchev	
Material:	fb			dm	fb*	fb	
Replicate:	a	b	c			a	b
14:0	5.4	3.1	2.3	2.3	3.4	2.8	1.1
15:0	3.6	1.7	1.6	2.3	2.4	3.2	0.6
16:0	27.4	21.5	18.5	26.1	21.5	33.4	31.7
16:1(n-9)	24.7	11.5	16.5	25.8	24.9	19.4	1.5
16:1(n-7)	9.4	31.9	36.0	9.7	14.3	6.5	1.4
18:0	6.0	4.8	3.6	9.6	6.1	9.7	59.8
18:1(n-9)	16.0	8.5	6.3	14.2	10.7	15.6	1.9
18:1(n-7)	3.7	14.7	13.3	4.3	12.1	4.7	0.8
18:2(n-6)	0.6	0.6	0.4	0.9	0.8	1.1	0.0
20:0	0.6	0.3	0.3	0.3	0.8	0.0	0.7
20:1(n-9)	0.8	0.4	0.5	0.9	0.7	0.8	0.0
20:1(n-7)	0.6	0.0	0.0	0.8	0.5	0.6	0.0
20:5(n-3)	0.0	0.0	0.0	0.0	0.3	0.0	0.0
22:0	0.4	0.5	0.3	1.1	0.5	1.0	0.2
22:1(n-9)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5(n-3)	0.0	0.0	0.0	0.4	0.0	0.0	0.0
22:6(n-3)	0.8	0.5	0.4	0.8	0.8	0.9	0.1
24:1(n-9)	0.0	0.0	0.0	0.5	0.2	0.3	0.1
Saturated	43.4	31.9	26.6	41.7	34.7	50.1	94.3
Monounsaturated	55.2	67.0	72.6	56.2	63.4	47.9	5.6
Diene	0.6	0.6	0.4	0.9	0.8	1.1	0.0
PUFA	0.8	0.5	0.4	1.2	1.1	0.9	0.1

Table 3. *Rimicaris exoculata*. Fatty acid and fatty alcohol composition (%) of the major lipid classes in 'orange' (16 to 18 mm) and small adult (26 to 28 mm) *R. exoculata* containing predominantly wax ester neutral lipid reserves

Site:	Snake Pit						Logatchev					
Total length:	18 mm			28 mm			16 mm			26 mm		
Tissue:	Entire			Abdomen			Entire			Abdomen		
Lipid class:	Polar	Wax ester acid	Wax ester alcohol	Polar	Wax ester acid	Wax ester alcohol	Polar	Wax ester acid	Wax ester alcohol	Polar	Wax ester acid	Wax ester alcohol
14:0	0.5		1.0	0.3		0.9			1.8			0.9
16:0	13.8	0.1	46.5	12.5	0.2	44.3	11.0	0.4	48.4	12.2	0.2	45.1
16:1(n-9)	0.3	0.1	0.3	0.2		0.2	0.0	0.3	0.6		0.2	0.2
16:1(n-7)	5.4	11.1	1.3	3.5	10.5	1.6	3.7	18.6	2.2	2.8	13.8	1.6
17:0		0.4			0.3		0.4	1.3			0.4	
16:4	0.9						1.0			0.7		
18:0	3.6		9.7	3.5		9.2	3.7		9.3	3.1		8.8
18:1(n-9)	13.9	12.4	16.5	12.2	20.9	19.5	11.0	23.4	15.3	10.0	26.1	19.2
18:1(n-7)	16.5	8.6	21.6	20.8	0.6	21.9	20.7	1.8	20.0	24.0	0.9	21.2
18:2(n-6)	0.8	1.8		0.7	2.0		0.7	1.5		0.5	1.7	
18:2(n-4)				0.2			0.3					
18:3(n-3)		0.8			0.5			1.0			0.4	
18:4(n-3)	0.8	4.1			2.9			0.9			2.7	
20:0	0.2		0.2			0.2			0.3			0.2
20:1(n-9)	0.8	0.1	2.8	0.4		2.3	0.3	0.2	2.0			2.7
20:2Δ5, 13				0.3			0.3					
20:4(n-6)	2.3	3.2		2.2	3.9		6.0	5.2		1.9	4.7	
20:4(n-3)	0.3	0.8		0.2	0.6		0.1	0.5		0.3	0.8	
20:5(n-3)	17.0	16.5		17.5	19.2		15.1	8.1		17.1	14.4	
22:2Δ7, 15	0.8			0.7			1.4			0.6		
22:5(n-6)	0.5	3.7		0.6	3.9		1.7	10.1		0.6	5.1	
22:5(n-3)	0.6	2.0		0.9	1.8		0.8	1.7		1.2	2.0	
22:6(n-3)	19.7	34.2		22.0	32.9		21.3	25.0		24.8	26.6	
24:1(n-9)	1.4	0.0		1.1			0.4					
Saturated	18.0	0.5	57.5	16.3	0.5	54.5	15.1	1.7	59.8	15.3	0.6	55.1
Mono-unsaturated	38.3	32.4	42.5	38.2	32.0	45.5	36.1	44.3	40.2	36.8	41.0	44.9
Diene	1.6	1.8		1.9	2.0		2.7	1.5		1.1	1.7	
PUFA	42.1	65.3		43.4	65.5		46.1	52.3		46.8	56.7	

acids in the juvenile shrimp were 20:5(n-3), 18:1(n-7), 18:1(n-9), 16:1(n-7) and 16:0. The fatty alcohol components of the wax esters comprised almost exclusively 16:0, 18:1(n-7) and 18:1(n-9) fatty alcohols (Table 3). The fatty acid composition of the small adult *R. exoculata* was broadly similar to that of the juvenile shrimp, although 22:6(n-3) comprised a higher proportion of fatty acids and was a very substantial component of the wax ester and triacylglycerol lipids (50.2 and 52.1% respectively, Table 4).

Adult *Rimicaris exoculata* contained minor amounts of wax ester, and thus most fatty acids were contained within the polar and triacylglycerol lipid classes. There were some fundamental differences in the fatty acid profiles of adult shrimp compared with the juvenile and small adult shrimp, with lower proportions of the (n-3) PUFAs 20:5(n-3) and 22:6(n-3). This was particularly evident in triacylglycerols where in some cases only very low levels of (n-3) PUFA were found (Table 5). The fatty acid profiles of adult *R. exoculata* also differed in that they contained substantial amounts of (n-4) and (n-7) fatty acids and also the nonmethylene interrupted dienes 20:2Δ5, 13 and 22:2Δ7, 15 (Table 5).

δ¹³C values

Stable carbon isotope values of individual fatty acids were highly variable with values ranging from $-6.6‰$ for 18:2(n-4) for the 57 mm adult specimen taken from Broken Spur to $-29.1‰$ for 16:1(n-7) in the 53 mm adult from the Rainbow site. Although $\delta^{13}C$ values for fatty acids within single shrimp were less variable, there were still substantial differences of up to 11.5‰ (53 mm adult from Rainbow, Table 6). $\delta^{13}C$ values of 20:5(n-3) and 22:6(n-3) in all categories of shrimp varied from -17.1 to $-27.1‰$. $\delta^{13}C$ values of the fatty alcohols were less variable with overall maximum and minimum values of -21.0 and $-26.7‰$ respectively (Tables 7 & 8).

Table 4. *Rimicaris exoculata*. Fatty acid and fatty alcohol composition (%) of small adults containing both wax ester and triacylglycerol neutral lipid reserves

Site:	TAG							
Total length:		26 mm				25 mm		
Tissue:		Abdomen				Abdomen		
Lipid class:	Polar	Triacyl-glycerol	Wax ester acid	Wax ester alcohol	Polar	Triacyl-glycerol	Wax ester acid	Wax ester alcohol
14:0		0.3		0.5		2.1		2.8
14:1						0.4		
15:0		0.2						
16:0	10.1	6.4	0.8	42.3	11.4	13.8	0.5	46.7
16:1(n-9)		1.1	0.3	0.2		1.2	0.6	0.4
16:1(n-7)	7.2	4.7	2.1	1.1	4. 6	13.7	20.4	1.8
16:1(n-5)			1.1			1.4		
16:2(n-4)	1.4	1.0				7.0		
17:0			1.1				0.7	
16:4					1.0			
18:0		1.6	0.7	14.5	4.5	2.7		9.9
18:1(n-9)	12.1	4.6	4.3	16.0	13.5	13.9	25.5	16.7
18:1(n-7)	19.6	4.1	0.4	22.7	22.5	10.2	1.9	18.5
18:2(n-6)	0.9	0.4	0.3		1.0	0.7	2.1	
18:2(n-4)	6.9	4.4			1.5	10.9		
18:3(n-7)	1.8					0.7		
18:3(n-3)							1.4	
18:4(n-3)							0.7	
20:0				0.5				0.4
20:1(n-9)	0.3			2.2				2.6
20:2Δ5, 13	1.1	0.4						
20:4(n-6)	8.9	4.8	5.9		2.1	1.4	4.8	
20:4(n-3)			0.2			0.2	0.3	
20:5(n-3)	9.7	4.5	11.7		14.6	4.0	8.3	
22:1								
22:2Δ7, 15	1.1				1.1	0.4		
22:5(n-6)	1.8	6.9	17.4			0.9	7.0	
22:5(n-3)	0.7	2.5	3.6			0.6	1.5	
22:6(n-3)	16.4	52.1	50.2		21.8	13.7	24.3	
24:1(n-9)					0.6			
Saturated	10.1	8.5	2.6	57.8	15.9	18.6	1.2	59.8
Monounsaturated	39.2	14.5	8.2	42.2	41.2	40.8	48.4	40.2
Diene	11.4	6.2	0.3		3.6	19.0	2.1	
PUFA	39.3	70.8	89.0		39.5	21.5	48.3	

DISCUSSION

Deep sea hydrothermal vent environments support abundant, albeit highly localized, animal communities (Childress & Fisher 1992, Van Dover 1995). The super-heated fluids discharged at vent sites are rich in geo-thermally reduced compounds, primarily hydrogen sulfide and methane, which are oxidized by chemoautotrophic bacteria to provide an energy source to drive the fixation of CO_2 and other C_1 compounds into organic molecules. The vent animal community then uses the organic metabolites produced by these bacteria as food. Since their discovery, it has generally been accepted that deep sea hydrothermal vent ecosystems are not reliant on processes occurring in the surface layers of the ocean and that the contribution of organic molecules fixed by

photosynthetic processes is negligible. However, recent biomarker and stable isotope evidence suggests that some animals associated with the East Pacific (Rieley et al. 1995) and MAR vent sites (Pond et al. 1997a,b,c) can contain high levels of compounds which originate in photosynthetic microplankton.

The life-history strategy of MAR vent shrimp is now reasonably well understood (Dixon et al. 1998, Herring 1998). It is clear that these animals spend a substantial period of their early life stages as planktotrophic organisms, feeding on photosynthetic material and accumulating substantial reserves of neutral lipid before returning to a suitable vent site, prior to maturation (Pond et al. 1997a, Dixon et al. 1998). In the present study, analyses of bacterial/detrital material collected from the vent sites and at the same locations as the

Table 5. *Rimicaris exoculata*. Fatty acid composition (%) of adults which contain only triacylglycerol neutral lipid reserves

Site: Total length: Tissue: Lipid class:	Rainbow 53 mm Maxilliped Polar	Rainbow 53 mm Maxilliped Triacylglycerol	Rainbow 20.7 mm Abdomen Polar	Rainbow 20.7 mm Abdomen Triacylglycerol	Broken Spur 57 mm Abdomen Polar	Broken Spur 57 mm Abdomen Triacylglycerol	TAG 26 mm Maxilliped Polar	TAG 26 mm Maxilliped Triacylglycerol	Snake Pit 57 mm Abdomen Polar	Snake Pit 57 mm Abdomen Triacylglycerol	Logatchev 29 mm Abdomen Polar	Logatchev 29 mm Abdomen Triacylglycerol
14:0		1.0		0.8		1.6		4.9	0.2	0.8	0.2	0.7
14:1		0.4		0.3		0.7		5.3				
15:0		0.3		0.3								
16:0	12.4	14.1	9.9	17.4	9.7	11.2	8.1	8.9	9.8	11.5	9.5	12.0
16:1(n-9)		1.0		0.7		0.2			0.1	1.5		0.6
16:1(n-7)	15.1	21.5	15.6	28.9	15.2	24.3	18.4	21.2	6.1	10.2	9.9	16.3
16:1(n-5)								2.8				1.2
16:2(n-4)	0.6	2.6	0.7	3.5	1.5	5.0	8.2	15.6	0.5	2.5	1.7	6.6
17:0												
16:4	0.9			0.9				1.2		0.9		
18:0	3.0	2.0		1.3	2.2	1.8	3.5	0.8	5.5	2.8	4.3	3.4
18:1(n-9)	8.8	11.0	11.8	12.8	6.4	2.9	5.4	4.3	7.4	7.8	7.7	5.4
18:1(n-7)	21.3	10.7	22.6	12.8	21.5	13.5	15.6	10.0	19.7	10.0	18.2	14.2
18:2(n-6)	0.2	0.4	0.5	0.2	1.5	0.1	0.5	0.1	0.4	0.5	1.2	0.2
18:2(n-4)	10.4	8.6	11.5	12.2	14.3	24.8	16.7	19.7	4.5	8.2	13.1	25.2
18:3(n-7)	0.8	0.4	2.4		9.2	8.6	0.8		0.9	1.7	5.8	7.8
20:0		0.3	0.4								0.2	
20:1			0.6	0.5	0.7		0.4	0.5	0.5		0.8	0.3
20:2Δ5, 13	3.3	2.4	7.9	4.9	4.9	3.3	0.3	0.4	0.5		2.7	1.8
20:4(n-6)	1.7	2.0	3.2	0.1	0.7	0.4	5.5	0.5	5.4	7.8	1.5	0.4
20:4(n-3)		0.2							0.2	0.5		
20:5(n-3)	7.9	3.9	5.8	0.5	4.0	0.5	6.4	0.8	14.1	8.8	8.4	1.3
22:1							0.2					
22:2Δ7, 15	1.0	0.6	1.7	0.9	1.4	0.4	0.9	0.7	0.9		0.9	0.3
22:5(n-6)	0.5	1.4	0.5	0.2	0.6		0.8	0.6	1.7	13.1	0.7	0.2
22:5(n-3)	0.3	0.6	0.4		0.4		0.3	0.2	0.8	4.0	0.7	
22:6(n-3)	11.8	14.5	4.5	1.3	4.8	0.7	8.1	2.4	19.8	8.4	11.4	2.1
24:1(n-9)		0.2		0.3				0.1			0.2	
Saturated	15.4	17.7	10.3	19.8	11.9	14.6	11.6	14.6	15.5	15.1	14.2	16.1
Monounsaturated	45.2	44.4	50.6	56.3	43.8	40.9	40.0	44.2	33.8	29.5	36.8	38.0
Dienes	15.5	14.6	22.3	21.7	23.6	33.6	26.6	36.5	6.8	11.2	19.6	34.1
PUFA	23.9	23.0	16.8	2.1	20.6	10.9	21.9	4.5	42.9	44.3	29.8	11.8

Table 6. *Rimicaris exoculata*. Stable carbon isotope values (δ^{13}C ‰ v-PDB) of the fatty acids and fatty alcohols contained in the major lipid classes of adults

Site: Total length: Tissue: Lipid class:	Rainbow 53 mm Maxilliped Polar	Rainbow 53 mm Maxilliped Triacylglycerol	Rainbow 20.7 mm Abdomen Polar	Rainbow 20.7 mm Abdomen Triacylglycerol	Broken Spur 57 mm Abdomen Polar	Broken Spur 57 mm Abdomen Triacylglycerol	TAG 26 mm Maxilliped Polar	TAG 26 mm Maxilliped Triacylglycerol	Snake Pit 57 mm Abdomen Polar	Snake Pit 57 mm Abdomen Triacylglycerol	Logatchev 29 mm Abdomen Polar	Logatchev 29 mm Abdomen Triacylglycerol
16:0	-20.2	-18.8	-21.2	-17.8	-12.1	-14.0	-17.2	-16.0	-16.2	-22.8	-15.7	-16.9
16:1(n-7)	-29.1	-14.5	-11.2	-12.4	-8.4	-8.8	-12.4	-13.5	-17.6	-22.3	-12.8	-13.1
16:2(n-4)		-16.8	-10.4	-12.5	-7.9	-8.8	-11.2	-13.8	-17.5	-21.4	-14.1	-14.7
18:0		-22.3		-20.5	-11.2	-13.2						-18.5
18:1(n-9)	-19.1	-21.3	-18.2	-18.4	-12.1	-11.1	-17.4	-19.1	-18.9	-24.6	-18.7	-20.3
18:1(n-7)	-17.7	-13.7	-17.4	-11.7	-10.2	-9.2	-11.5	-15.7	-18.5	-21.3	-14.3	-14.6
18:2(n-4)		-14.5	-11.1	-11.8	-11.1	-6.6	-12.1	-14.8	-17.7		-11.7	-13.7
20:2Δ5, 13		-16.8	-16.7	-17.9	-10.3	-11.0						
20:4(n-6)							-19.5		-22.1	-21.2		
20:5(n-3)	-17.6	-17.8	-18.1		-18.4		-19.2		-18.3	-20.4	-20.3	
22:5(n-6)										-23.3		
22:6(n-3)	-22.4	-22.3	-21.7		-17.1		-16.8		-24.2	-19.5	-19.6	

Table 7. *Rimicaris exoculata*. Stable carbon isotope values ($\delta^{13}C$ ‰ v-PDB) of fatty acids and fatty alcohols contained in the major lipid classes of 'orange' (16 to 18 mm) and small adults (26 to 28 mm) [*indicates where 18:1(n-9) and 18:1(n-7) are unresolved]

Site:	Snake Pit						Logatchev					
Total length:	18 mm			28 mm			16 mm			26 mm		
Tissue:	Entire			Abdomen			Entire			Abdomen		
Lipid class:	Polar	Wax ester acid	Wax ester alcohol	Polar	Wax ester acid	Wax ester alcohol	Polar	Wax ester acid	Wax ester alcohol	Polar	Wax ester acid	Wax ester alcohol
16:0	−20.2		−22.0	−18.9		−21.9	−20.4		−24.8	−20.3		−21.0
16:1(n-7)	−29.0	−23.9		−19.7	−20.9			−22.3		−21.2	−19.4	
18:0			−23.2			−21.5			−23.2			−25.0
18:1(n-9)	−19.1	−18.8	−21.7	−18.4*	−20.2	−26.0	−22.6*	−20.3	−23.8	−20.4*	−21.1	−25.4
18:1(n-7)	−17.7	−15.2	−21.8			−24.0		−20.0	−23.3			−21.9
20:4(n-6)					−25.9		−18.3					
20:5(n-3)	−21.4	−21.2		−22.3	−23.8		−21.1	−19.8		−20.5	−20.0	
22:6(n-3)	−22.4	−20.4		−21.9	−27.1		−21.7	−19.9		−22.1	−19.3	

Table 8. *Rimicaris exoculata*. Stable carbon isotope values ($\delta^{13}C$ ‰ v-PDB) of the major fatty acids and fatty alcohols in small adults

Site:	TAG							
Total length:	26 mm				25 mm			
Tissue:	Abdomen				Abdomen			
Lipid class:	Polar	Triacyl-glycerol	Wax ester acid	Wax ester alcohol	Polar	Triacyl-glycerol	Wax ester acid	Wax ester alcohol
16:0	−20.2	−22.2	−27.3	−23.0	−23.6	−24.1	−29.0	−25.4
16:1(n-7)	−17.2	−18.7	−25.8		−20.8	−22.3	−26.3	
18:0	−24.3	−26.2	−28.0	−26.7				−25.6
18:1(n-9)	−19.2	−22.0	−25.2	−24.0	−20.6	−21.5	−23.2	−25.6
18:1(n-7)	−21.5	−20.9	−23.8	−24.8	−17.0	−17.6	−20.8	−26.7
18:2(n-4)	−18.2	−18.4				−17.2		
20:4(n-6)	−20.5	−23.7	−21.1		−24.8	−25.6	−24.8	
20:5(n-3)	−22.6	−24.7	−25.5		−22.6	−24.7	−23.9	
22:5(n-6)		−23.7	−23.3				−22.7	
22:6(n-3)	−17.9	−17.6	−22.6		−20.7	−23.1	−22.6	

samples of shrimp showed only very low amounts of PUFA which supports the contention that these same compounds in the shrimp are derived from photosynthetic microplankton.

The detailed analyses of the neutral lipid reserves contained in the juvenile and small adult *Rimicaris exoculata* have established that they contain extraordinarily high levels of PUFA (up to 89% of total fatty acids, Tables 3 to 5). In marine animals which are known to accumulate triacylglycerol or wax esters as reserve lipids, the fatty acids comprising these compounds are predominantly saturated (no double bonds) or monounsaturated fatty acids (a single double bond) and PUFA are often only minor components (Sargent & Henderson 1995). Thus, the high levels of PUFA contained in the neutral lipids of *R. exoculata* are considerably higher than those previously reported for marine Crustacea (Falk-Petersen et al. 1987, Norrbin et al. 1990, Sargent and Henderson 1995, Kattner et al.

1996). GC-IRMS analyses indicate that the $\delta^{13}C$ values of these PUFA contained in neutral lipid are from −17.6 to −27.1‰, which is within the range consistent with a photosynthetic origin of these compounds and supports previous studies (Pond et al. 1997a,b,c, in press). We believe that these PUFA are selectively accumulated by the shrimp during their early planktonic life stage before subsequent mobilization into phospholipids as and when required to sustain growth and maturation of the shrimp into adulthood. This selective incorporation of dietary PUFA into wax ester reserves during the planktotrophic life stage must ultimately be genetically regulated, which indicates that *R. exoculata* has evolved a highly specialized lipid metabolism adapted to its life cycle.

There is a clear progression from a lipid profile in the juvenile shrimp which is dominated by wax esters and PUFA, to the small adult which contains both wax ester and triacylglycerol and finally to the adult shrimp with

triacylglycerol and the presence of bacterial signature fatty acids [16:2(n-4), 18:2(n-4); 20:2Δ5, 13; 22:2Δ7, 15]. This progression in the lipid composition is entirely consistent with the 'orange' shrimp being a juvenile stage of *Rimicaris exoculata*. The transition between a neutral lipid profile which is dominated by wax esters during the planktotrophic stage and triacylglycerols after maturation (Fig. 1, and Copley et al. 1998) is unusual as most lipid-rich marine animals tend to store a single neutral lipid class throughout their life cycle. Wax esters are considered to be long term metabolic energy reserves and are characteristic of animals which experience marked seasonality in the availability of their food (Lee & Hirota 1973). Wax ester reserves have also been implicated in providing buoyancy which would be beneficial for planktonic animals as it could plausibly reduce the metabolic costs associated with swimming (Sargent 1976). Although the specific gravity of lipid is lower than unity, there are considerable differences between the various lipid classes. The specific gravity of wax ester is approximately 0.86 which would provide an upthrust of 0.193 g g^{-1} lipid (Sargent 1976). Triacylglycerols, with a specific gravity of 0.93, would provide an upthrust force of only 0.103 g g^{-1} lipid and may therefore be the preferred neutral reserve lipid for a benthic animal which swims and feeds immediately adjacent to buoyant hydrothermal plumes. However, it is clear that whatever the reason for the transition from wax ester to triacylglycerol storage lipids during maturation, the mobilization of PUFA from wax ester into triacylglycerols again points to a highly regulated and evolved lipid metabolism.

It is not within the scope of this study to compare differences in the lipid and stable carbon isotope values of shrimp between vent sites, but it is obvious that the isotope signatures of the fatty acids are highly variable, as has previously been observed for biological material collected from hydrothermal vent fields (Fisher et al. 1994, Van Dover & Fry 1994, Pond et al. 1997c). The $\delta^{13}C$ values of the fatty alcohols were generally less variable than those of the fatty acids. The ranges of $\delta^{13}C$ values in photosynthetic systems are usually much less variable than those in chemoautotrophic organisms (Goericke et al. 1994), which suggests that the fatty alcohols are derived from a photosynthetic carbon source.

Given the widespread distribution of *Rimicaris exoculata* along the MAR, coupled with their potentially high population densities (Gebruk et al. 1997), these animals must be responsible for a substantial and direct transport of PUFA into the hydrothermal vent ecosystem. The wider ecological implications of this 'import' of PUFA into the vent ecosystem are unknown. Organisms such as MAR vent mussels appear not to require high levels of PUFA, as their tissues contain

only very low amounts of these compounds (Pond et al. 1998). However, any fish populations associated with the MAR vent sites would undoubtedly require a dietary input of PUFA as these compounds are essential for vertebrate nutrition (Sargent et al. 1995).

In summary, it is clear from the above findings that the life-history strategy and nutritional biochemistry of *Rimicaris exoculata* are highly specialized and have evolved specifically to enable this shrimp species to obtain and store substantial amounts of PUFA produced by phytoplankton. Thus, although these shrimp are similar to the vast majority of other animals which inhabit the marine environment, in that they are to a degree dependent on organic compounds produced in the surface layers of the ocean, they are also able to exploit successfully the abundant chemosynthetic food resources available at the deep water MAR vent sites.

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